Lexis and grammar of mitochondrial RNA processing in Trypanosomes

Trends in Parasitology

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--Manuscript Draft--

Manuscript Number:	
Article Type:	Review
Keywords:	Trypanosoma; mitochondria; kinetoplast; RNA editing; RNA decay; polyadenylation; translation.
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Abstract:	Parasitic protist Trypanosoma brucei sp. cause African human and animal trypanosomiasis. These hemoflagellates belong to the class Kinetoplastea, a taxon distinguished by the presence of a kinetoplast. This nucleoprotein body contains interlinked circular DNAs of two kinds. The maxicircles encode 9S and 12S rRNAs, two guide RNAs (gRNAs), and 18 proteins. The minicircles bear gRNA genes. Both maxicircle and minicircle genomes are transcribed from multiple promoters into 3′ extended precursors which undergo 3′-5′ exonucleolytic trimming. For translation, most pre-mRNAs further proceed through 3′ adenylation, and often undergo U-insertion/deletion editing, and 3′ A/U-tailing. The rRNAs and gRNAs are typically 3′

Highlights

- Mitochondrial RNA processing events in kinetoplastid protists include 5′ modifications, 3′-5′ degradation, internal sequence changes by U-insertion/deletion mRNA editing, and non-templated 3′ extensions.
- The specificity of mRNA editing is dictated by guide RNAs while 5′ modifications and 3′ extensions are controlled by diverse PPR RNA binding factors.
- Antisense transcription plays a central role in delimiting 3′-5′ trimming of primary transcripts.
- Macromolecular protein and ribonucleoprotein complexes and auxiliary factors involved in these processes have been identified and characterized to varying degrees. This review discusses recent developments and introduces a consensus nomenclature for mitochondrial RNA processing complexes and factors in *T. brucei*.

Outstanding Questions

- Recent understanding that mitochondrial mRNA, gRNA and rRNA genes are transcribed individually as 3′ extended precursors places the onus on timing and mutual dependence of RNA synthesis and processing events. The nature of mitochondrial promoters and terminators, the composition of the transcription complex, the functionality of its components and the precise role of antisense transcripts remain to be elucidated.
- Many RESC subunits lack recognizable motifs and yet several bind RNA and most are essential for editing and cell viability. Understanding their functions would be a feast for structural biology.
- The mitochondrial ribosome likely selects fully-edited A/U-tailed mRNAs, but the mechanism of translation initiation at the 5′ end is unclear.
- Mitochondrial proteomics remain a challenge due to extremely hydrophobic nature of mitochondrially-encoded proteins.
- The stage-specific patterns of mitochondrial transcription, RNA processing and translation are likely coordinated with nuclear gene expression by yet unknown mechanisms.

Lexis and Grammar of Mitochondrial RNA Processing in Trypanosomes

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Abstract

Parasitic protist *Trypanosoma brucei* sp. cause African human and animal trypanosomiasis, a spectrum of diseases affecting the population and economy in sub-Saharan Africa. These dixenous hemoflagellates belong to the class Kinetoplastea, a taxon distinguished by the presence of a kinetoplast. This nucleoprotein body contains mitochondrial DNA of two kinds: maxicircles and minicircles. The maxicircles (~25 per genome, each ~23 kb) encode 9S and 12S ribosomal RNAs, two guide RNAs (gRNAs), two ribosomal proteins and 16 subunits of respiratory complexes. The minicircles (~5,000 per genome, ~1 kb each) bear gRNA and gRNA-like small RNA genes. Relaxed maxicircles and minicircles are interlinked and packed into a dense disc-shaped network by association with histone-like proteins. Both maxicircle and minicircle genomes are transcribed by a T3/T7 phage-like RNA polymerase from multiple promoters into 3′ extended precursors which undergo 3′-5′ exonucleolytic trimming. To function in mitochondrial translation, most pre-mRNAs must further proceed through 3′ adenylation, and often undergo gRNA-directed uridine insertion/deletion editing, and 3′ A/U-tailing. The ribosomal and guide RNAs are typically 3′ uridylated. Historically, the fascinating phenomenon of RNA editing has attracted major research effort, and recent developments provided insights into pre- and post-editing processing events and identified key players in transforming primary transcripts into mature RNAs, and regulating their function and turnover. Here, we introduce a unified nomenclature of mitochondrial RNA processing complexes and factors in *T. brucei* and attempt to integrate known modalities of kinetoplast RNA metabolism.

Mitochondrial Gene Expression in Trypanosomes: A Trove of Unconventional Biology

Protist parasites of the genus *Trypanosoma* have occupied the research spotlight since 1895 when David Bruce identified *Trypanosoma brucei* as the causative agent of animal trypanosomiasis (Nagana) [1], and later works linked these organisms to sleeping sickness in humans [2]. Biomedical, economic and societal impact of parasite infections warranted in-depth studies of the fascinating biology underlying *T. brucei* metabolism, development and interactions with insect vector and mammalian hosts [3]. Among the most striking cellular features of these parasites is the bipartite mitochondrial genome consisting of maxicircles and minicircles, and aggregately referred to as the kinetoplast DNA (kDNA). In *T. brucei*, maxicircles are catenated with minicircles into a single network and compacted by histone-like basic proteins. The progress in elucidating kDNA maintenance and segregation has been reviewed elsewhere [4-6]. Maxicircles, an equivalent of mitochondrial genomes (mtDNA) in other organisms, encode 9S and 12S rRNAs, two ribosomal proteins [7], and 16 subunits of respiratory complexes. Unlike most organellar genomes, kDNA lacks tRNA genes [8, 9], and 12 maxicircle genes are present as cryptogenes whose transcripts require RNA editing to restore a protein-coding capacity [10]. The editing is mediated by hundreds of guide RNAs (gRNAs) which are mostly encoded by minicircles, with only two gRNAs encoded by maxicircles. The distinct, albeit interlinked, maxicircle and minicircle genomes are transcribed independently, but the information converges at the post-transcriptional level whereby minicircle-encoded gRNAs direct editing of maxicircle encoded pre-mRNAs. The evolution of editing and whether this process confers a selective advantage to kinetoplastids remain the subject of a debate [11], but the existence of alternatively edited mRNA sequences and cognate gRNAs raises a possibility that editing-driven protein diversity may be functionally relevant [12-15]. Historically, much attention has been focused on RNA editing

mechanism and composition of editing complexes [16-22] while more recently major advances have been made in understanding transcription [23], primary RNA nucleolytic processing [24-26], 5′ [23] and 3′ modifications [27-32], and ribosome biogenesis and translation processes [7, 33-35]. The perceived complexity of mitochondrial gene expression has been exacerbated by recent influx of new RNA processing factors and numerous names often referring to the same entity. Bearing in mind that functions of only few proteins and complexes are established beyond reasonable doubt, we nonetheless submit that the process of discovering major players is close to completion. Here, we outline major stages in kinetoplast RNA processing and build on previous attempts [36, 37] to introduce a unified nomenclature for respective protein and ribonucleoprotein complexes, enzymes and factors in *T. brucei*. Given that orthologs of nearly all *T. brucei* proteins listed in Table 1 are readily distinguishable in related organisms, this nomenclature should be broadly applicable to other *Kinetoplastea*.

Nucleolytic Processing of Primary Transcripts

In most organisms, primary polycistronic mtDNA transcripts are punctuated by tRNAs whose excision by RNases P and Z defines functional RNA boundaries [38]. Although loss of mtDNA-encoded tRNA genes renders such a mechanism inapplicable to *T. brucei*, it has been held that mature mRNAs and rRNAs with uniformly monophosphorylated 5′ and well-demarcated 3′ termini reflect maxicircle polycistronic precursor's partitioning by an unknown endonuclease [39, 40]. To that end, a prominent transcription start site has been mapped within the divergent region ~1,200 nt upstream of 12S rRNA [41] and transcription proceeding through intergenic regions has been reported [42]. The uridylated rRNA [43] and adenylated mRNA [44] termini also typify distinct 3′ end modification mechanisms for these

RNA classes. Conversely, short (30-60 nt) gRNAs maintain 5′ triphosphates characteristic of transcription-incorporated initiating nucleoside triphosphate and, similar to rRNAs, are 3′ uridylated [45]. However, the only explored candidate gRNA precursor processing endonuclease KRPN1 (mRPN1) [46] is dispensable for axenically grown bloodstream stage parasites [42]. Thus, it may be argued the essentiality of RNA editing [47], which requires mature gRNAs [25, 48], renders KRPN1 an unlikely contributor to gRNA precursor processing. The observations conducive to the endonucleolytic model have been re-examined in light of mitochondrial 3′ processome (MPsome) discovery [24]. These studies recognized the MPsome-catalyzed 3′-5′ exonucleolytic degradation as the major nucleolytic processing pathway for mRNA and rRNA [29], and for gRNA [24] precursors. Composed of KRET1 TUTase [49], KDSS1 exonuclease [26, 50] and mitochondrial processome subunits (MPSS) 1-6 (Table 1), the purified MPsome displays 3′-5′ RNA degradation, RNA hydrolysis-driven double-stranded RNA unwinding, and 3′ RNA uridylation activities. Although the autonomous KDSS1 is inactive, incorporation into the MPsome converts this polypeptide into a highly processive exonuclease capable of degrading structured RNAs to 5-6 nt fragments. On the other hand, individual KRET1's robust UTP polymerization activity [51] is tamed upon MPsome assembly to adding 1-15 Us, a pattern consistent with the U-tails observed in steady-state RNA [15, 52]. Cumulatively, detection of *in vivo* uridylated precursors and degradation intermediates [24, 31], stimulation of *in organello* KRET1-dependent RNA decay by UTP [27], and MPsome's preference for U-tailed substrates suggest that uridylation by KRET1 activates RNA degradation by KDSS1. It is unclear whether substrate tunneling occurs within the same particle, but a coupling between RNA uridylation and degradation by 3'-5' RNase II/RNB-type exonuclease appears to be a highly conserved and phylogenetically widespread mode of RNA decay [53, 54].

Exonucleolytic processing is often a case of regulated decay whereby mature 3′ termini are defined by a degradation blockade at a specific sequence or structure. In the *T. brucei* mitochondrion, antisense transcripts cause MPsome pausing at 10-12 nt before double-stranded region at which point MPsome-embedded KRET1 likely adds a U-tail causing disengagement from the RNA [23, 24]. It follows that the precise transcription initiation site on the antisense strand defines the position of mature 3′ terminus of the sense transcript. The antisense model of gRNA 3′ end definition is consistent with bi-directional transcription from converging promoters otherwise recognized as imperfect 18-nt inverted repeats [55] that flank almost all gRNA genes in *T. brucei* minicircles [56, 57]. Identification of gRNA-sized short antisense RNAs and accumulation of antisense precursors upon KRET1 and KDSS1 knockdowns [24] further indicates that sense and antisense precursors hybridize with their complementary 5′ regions. In the current model, the length of a double-stranded region, which is a distance between respective transcription start sites, likely defines gRNA length prior to uridylation [24]. However, most *T. brucei* minicircles encode 3-4 gRNA cassettes [57] and primary RNAs may exceed a 1 kb linear length of a minicircle [24, 25]. Hence, an extensive transcription of both strands may also generate much longer double-stranded RNAs that are degraded by yet unknown mechanism. Conversely, *L. tarentolae* minicircle typically contains a single gRNA gene and lacks recognizable inverted repeats [58]. Although both strands are transcribed [59], the gRNA-flanking sequences are dissimilar from those of *T. brucei*, which indicates a divergent nature of minicircle promoters among trypanosomatids. The maxicircle promoters remain to be determined, but detection of antisense transcription start sites near intergenic regions and presence of corresponding non-coding antisense transcripts make a reasonable argument for a general mechanism of 3′ end definition for minicircle and maxicircle transcripts [23].

Modification of the 5′ End

The 5′ monophosphorylated termini of maxicircle-encoded rRNAs and mRNAs have long been interpreted as indicative of endonucleolytic partitioning of polycistronic precursors. It is, however, unfeasible to produce more than one monocistronic mRNA from a precursor by 3′-5′ degradation. This logic dictates that: 1) each gene rests under control of a dedicated promoter; 2) the 5′ terminus is set by transcription initiation; 3) inorganic pyrophosphate (PPi) is selectively removed from initiating nucleoside triphosphate in mRNAs and rRNAs, but not in gRNAs; and 4) transcription may proceed across multiple genes and produce a 3′ extended precursor of which only the most 5′ coding region is preserved after 3′-5′ trimming. Identification of the 5′ pyrophosphate processome complex (PPsome) partially resolved the question of differential phosphorylation status and linked 5′ PPi removal to mRNA stability [23]. A stable protein complex of MERS1 NUDIX [Nucleoside diphosphate linked to (X)] hydrolase and MERS2 PPR RNA binding subunit, the MPsome selectively binds to degenerate G-rich motifs found near mRNA 5′ ends, but not in gRNAs. MERS1 hydrolase is catalytically inactive as an individual protein while MERS2 confers both binding specificity and affinity for RNA substrate. Remarkably, MERS1 downregulation or replacement with an inactive version effectively eliminates most mRNAs but exerts negligible effects on gRNAs and rRNAs. It appears that rRNA is stabilized by different factors, possibly those involved in ribosome biogenesis [35]. Although PPsome-dependent mRNA protection against 3′-5′ degradation (see below) and the essential role of PPi removal are evident, the mechanistic insights into these processes will likely come from understanding PPsome's interactions with RNA editing substrate binding (RESC) and polyadenylation (KPAC) complexes discussed below [32].

Modifications of the 3′ end

Non-templated 3′ nucleotide additions often wield profound influence on RNA processing, function, trafficking and turnover [60]. In *T. brucei*, mitochondrial RNA 3′ modifications are classified into U-tailing by KRET1 TUTase (gRNAs and rRNAs), A-tailing by KPAP1 poly(A) polymerase (most mRNAs [31]), and A/U-extensions which require both enzymes and a complex of Kinetoplast Polyadenylation Factors 1 and 2 (KPAF1/2, [30]). Lack of pronounced RNA substrate specificity for KRET1 and KPAP1 raises the question of accessory factors that enable modifications of distinct RNA classes, and the functionality of these extensions. The presence of short U-tails in gRNAs and rRNAs, as well as non-templated uridine residues sometimes found in mRNAs between the 3′ UTR and the A-tail [30], indicates that uridylation by the MPsome-embedded KRET1 is a default 3′ modification. It is plausible that U-tailing causes the MPsome to disengage from the precursor when degradation pauses near double-stranded region formed by antisense RNA. However, the U-tail itself does not exert an appreciable impact on mature gRNA or rRNA stability [25] and its functionality beyond termination of processing remains debatable [61-63]. Conversely, a short (15-30 nt) A-tail decorates most mRNAs and impacts stability depending on transcript's editing status [28, 31, 64]. As demonstrated by KPAP1 poly(A) polymerase loss-of-function studies [29, 31] and *in organello* decay assays [28], adenylation mildly de-stabilizes pre-edited transcripts only to become essential for maintaining RNAs that are edited beyond initial editing sites at the 3′ end. A short A-tail also stabilizes never-edited mRNAs (those that contain an encoded open reading frame and do not require editing). The coupling between an mRNA's editing status and opposing effects of adenylation points toward a surveillance system capable of both sensing the extent of internal U-insertions/deletions and enabling 3′ A-tail addition and function. In molecular terms, sequence-specific activators and inhibitors would be expected to modulate mRNA

adenylation by KPAP1, and the resistance of such a modified molecules to decay. The respective functions have been attributed to Kinetoplast Polyadenylation Factors (KPAF) 3 [29] and 4 [32], which belong to a family of 35 amino acid repeat-containing RNA binding (PPR) proteins. Discovered in land plants [65], the helix-turn-helix PPR motif recognizes a single nucleoside via side chains occupying cardinal positions 5 and 35 of the repeat (or the last position in a longer structure). An array of adjacent PPR repeats often folds into a superhelical domain capable of binding to specific RNA sequence and recruiting or blocking various enzymes [66-68]. In this context, KPAF3 reportedly binds to G-rich pre-edited mRNAs with sufficient affinity and coverage to stabilize these species following 3′-5′ trimming by the MPsome [29]. *In vitro* reconstitution experiments further demonstrate that KPAF3 stimulates KPAP1 polyadenylation activity and this effect depends on the presence of the G-rich site near the 3′ end. Remarkably, KPAF3 binding is eliminated by the initiating editing events leaving the stability of edited RNA reliant on A-tail added prior to editing [29]. Thus, KPAF3 functions as editing sensor and *bona fide* polyadenylation factor thereby connecting the internal sequence changes and 3′ modification [29-31].

The most apparent A-tail function would be protecting mRNA against degradation by the MPsome. However, *in vitro* studies show that A-tailed RNAs can be degraded by the purified MPsome, albeit less efficiently than uridylated substrates [24]. The A-tailed partially-edited pre-mRNAs are also somehow prevented from the post-editing addition of the 200-300 nt-long A/U-tail. This modification marks fully-edited molecules [31] and channels translationally-competent mRNA for translation [30, 33, 34]. Finally, the mechanism of mRNA stabilization by the PPsome must reconcile binding of this complex to the 5′ end with blocking 3′-5′ degradation [23]. To rationalize these observations, Mesitov et al., envisaged a *trans*-acting factor that recognizes a nascent A-tail to enable an interaction between PPsome occupying the 5′ end and polyadenylation complex (KPAC) bound to the 3′

end [32]. It has been proposed that the resultant circularization increases mRNA resistance to degradation and uridylation, and, therefore, to premature A/U-tailing and translational activation of partially-edited transcripts [32]. Trypanosomal genomes apparently lack mitochondrially-targeted canonical RRM motif-containing poly(A) binding proteins, but this function is fulfilled by KPAF4. This PPR protein is almost entirely composed of seven repeats of which five are predicted to bind sequential adenosine residues [69]. Co-purification studies support KPAF4 interactions with KPAC components (KPAP1, KPAF1/2) and RESC-mediated contacts with the PPsome. Accordingly, the A-tail has been identified as the predominant *in vivo* binding site while *in vitro* KPAF4 selectively recognizes adenylated substrates. Indeed, in the presence of KPAF4 adenylated RNA is more resistant to degradation by the purified MPsome and uridylation by KRET1 TUTase.

Although direct demonstration of mRNA circularization is lacking, this event can be imagined as a critical quality check point to ensure 5′ end occupancy by the PPsome and correct termination of 3′-5′ trimming downstream from the KPAF3 binding site. In this scenario, KPAF3 binding likely selects a correct 3′ UTR among trimmed precursor isoforms thereby distinguishing mRNA form rRNA and stimulating polyadenylation of the former by KPAP1. KPAF4 binding to a nascent A-tail would enable interaction with 5′ end-bound PPsome, hence, mRNA circularization. Consequentially, only A-tailed mRNAs would proceed though the editing cascade while the variants truncated beyond KPAF3 binding sites become uridylated and degraded [29]. It follows that upon editing completion at the 5′ end, a signaling event must take place to release the circularization and enable access of KPAF1/2 factors and KRET1 TUTase in order to add long A/U-tail to the pre-existing short A-tail. Although these postulates require further testing, it seems plausible that PPsome displacement from the 5′ end by final editing events may disrupt circularization and stimulate A/U-tailing, a final processing step that renders mRNA competent for translation.

U-insertion/deletion mRNA Editing

Editing Process

In *T. brucei*, six of the 18 annotated mRNAs encode predicted polypeptides while the remaining 12 transcripts undergo editing to acquire a protein-coding sequence. The extent of editing varies from minor, typified by insertion of four Us into three closely-spaced sites (COII mRNA, [10]), to moderate (e.g., *cyb* mRNA, 34 Us are inserted into a confined region near 5′ end [70]), to pan-editing during which hundreds of uridines are inserted or deleted throughout the entire transcript (e.g. ND7 mRNA, [71]). The determinants of position-specific U-insertions and deletions were discovered in the Simpson laboratory as short patches of complementarity between edited mRNA and maxicircle DNA in *Leishmania tarentolae* [45]. By allowing for G-U, in addition to canonical Watson–Crick base-pairing, short (50-60 nt) RNAs transcribed from minicircles have been recognized as carriers of genetic information and termed guide RNAs (gRNAs). *In vitro* experiments by the Stuart laboratory directly demonstrated that gRNAs indeed constitute the necessary and sufficient source of editing information [72-74]. Although the first gRNAs were discovered in the maxicircle, further work in *Leishmania* established that most gRNAs are encoded in minicircles [75]. In *T. brucei*, only two gRNAs have been identified in the maxicircle: a *cis*-acting element embedded into 3′ UTR of COII mRNA [76], and a *trans*-acting gRNA that completes editing of the MURF2 mRNA. The secondary structure of gRNA-mRNA dictates the editing site selection and the extent of U-insertions and deletions [72]. The initial gRNA-mRNA interaction is accomplished via a short (10-12 nt) region of complementarity between the gRNA's 5′ anchor region and the pre-edited mRNA. The remaining guiding segment forms an imperfect duplex with pre-mRNA resulting in looping out of single-stranded uridines in mRNA (deletion sites) or purine nucleotides in gRNA (insertion sites). At either site, the mRNA is cleaved at the first unpaired nucleotide adjacent to the 5′ anchor duplex.

The resultant structures of deletion and insertion intermediates are distinct: single-stranded uridines become exposed to a 3′-5′ exonucleolytic attack in the former, while a single-stranded gap is created between two helices in the latter. Upon trimming single-stranded uridines from the 5′ cleavage fragment in the deletion site or adding gap-specified number of Us into the insertion site, the fragments are joined to restore mRNA continuity. Both types of sequence changes extend the double-stranded anchor region. Pan-editing requires multiple overlapping gRNAs and there is a method to it: sequence changes directed by the initiating gRNA create a binding site for the next one to ensure an overall 3′-5′ polarity along the editing domain. However, editing may not always proceed strictly 3′ to 5′ as "mis-edited" junctions are present at the leading edge of editing in the majority of steady-state mRNAs [59, 77-79]. The role of junctions is not entirely understood, but they likely represent a mixture of regions that undergo re-editing to canonical edited sequence, dead-end products, or mRNAs with alternative non-canonical coding sequences [22].

A single editing domain may cover an entire mRNA [80], or an isolated region [71]. An individual gRNA can theoretically direct insertions and deletion at several closely spaced sites (editing block), but as editing progresses within the block, the interaction between gRNA and mRNA 5′ cleavage fragment is supported by fewer base pairs. Stabilizing the 5′ cleavage fragment-mRNA tethering by additional base pairing stimulates cleavage and the full editing cycle *in vitro* [81-83], but it is unclear how the problem of editing at distal sites within one block or across sequential blocks is solved *in vivo*. An active displacement of a gRNA with diminishing "3′ tether" by RNA helicase is among possible solutions that would enable binding of succeeding gRNA within a domain. Alternatively, the gRNA's U-tail may help stabilize interaction with an mRNA [61, 84], but the conclusive evidence for this event is yet to be obtained.

316 ***Elemental Editing Reactions***

317 Editing reactions are catalyzed by enzymes embedded into the ~20S (~800 kDa) RNA
318 Editing Catalytic Complex (RECC), a remarkable example of a modular assembly that
319 enables broad functionality on distinct RNA substrates [85-89]. A common core particle
320 consists of U-insertion (KRET2 TUTase, KREPA1 zinc finger protein and KREL2 RNA
321 ligase) and U-deletion (KREX2 exonuclease, KREPA2 zinc finger protein and KREL1 RNA
322 ligase) sub-complexes and six structural and/or RNA binding proteins (KREPA3, KREPA4,
323 KREPA5, KREPA6, KREPB4, KREPB5). The U-insertion and U-deletion sub-complexes
324 likely function independently [48, 90] while most of the remaining components are essential
325 for assembly and/or integrity of the entire core particle [91-94]. The core particle is shared
326 among at least three RECC isoforms distinguished by association with endonuclease
327 modules. Each module is composed of an RNase III endonuclease and a partner protein(s)
328 and is primarily responsible for recognition and cleavage of insertion and deletion sites. The
329 U-deletion sites are recognized by the RECC isoform with KREN1+KREPB8+KREX1,
330 while U-insertion sites are recognized by the RECC isoforms with KREN2+KREPB7 or
331 KREN3+KREPB6, which display distinct and overlapping specificities [95-99]. The
332 canonical RNase III catalytic domain typically forms a functional homodimer with two active
333 sites that introduce four cuts into both strands of a double-stranded RNA [100]. In contrast,
334 editing endonucleases appear to cleave only mRNA. It seems plausible that RNA hydrolysis
335 is restricted to a single cut by heterodimer formation between KREN1, KREN2 or KREN3,
336 and catalytically-inactive degenerate RNase III domains in KREPB4 or KREPB5 [101]. A
337 contribution of RNase III partner proteins KREPB8, KREPB7 or KREPB6 to modulating
338 cleavage activity is also possible [102, 103]. Binding of KREN1, KREN2 and KREN3
339 modules to a common core containing U-deletion, U-insertion and ligase activities highlights
340 RECC's modular nature [98, 104, 105], but the nature of interactions responsible for mutually

exclusive contacts between the core and distinct modules remains unclear. Cross-linking mass-spectrometry points to interactions involving RNase III domain dimerization between editing endonucleases with partner proteins KREPB6, B7 or B8, and core proteins KREPB4 and KREPB5 [102, 103, 106].

Within the common core, the U-deletion and U-insertion cascades are spatially separated by virtue of editing enzymes binding to zinc finger proteins, KREPA2 and KREPA1, respectively [90, 107-109]. KREX1 and KREX2 proteins possess exonuclease-endonuclease-phosphatase (EEP) catalytic domains and display single-stranded uridine-specific 3′-5′ exonuclease activity *in vitro* [108, 110]. However, their protein-protein interactions are remarkably distinctive: the essential KREX1 belongs to the KREN1 endonuclease module, and is responsible for the main U-deletion activity; the dispensable KREX2 probably represents a structural component of the U-deletion sub-complex [96, 106]. Fittingly, *L. tarentolae* KREX2 lacks a catalytic domain, but remains associated with the U-deletion sub-complex [85]. In the U-insertion sub-complex, KRET2 TUTase binds to KREPA1, which results in a mutual stabilization and stimulation of TUTase activity [48, 106, 111-113]. Selectivity of uridine incorporation is determined by KRET2's intrinsic specificity for UTP [114] rather than the nature of the opposing nucleotide in the gRNA. To that end, the +1U addition occurs equally efficient irrespective of the corresponding nucleotide in gRNA, but the +2U addition occurs only if the +1U forms a base pair with either adenosine or guanine. Consequentially, both purine bases in guiding positions direct U-insertions with similar efficiency [81, 111] . RNA editing ligases 1 and 2 (KREL1 and KREL2) have been identified as components of U-deletion and U-insertion sub-complexes, respectively [90, 106, 107]. Although spatial separation appears to suggest specialized roles, only KREL1, but not KREL2, is essential for cell viability [47, 115, 116].

366 ***Editosome Definition***

367 From the early reports of RNA ligase-containing complexes sedimenting in glycerol density
368 gradients as particles with apparent 20S to 50S values [117, 118], the quest for an elusive
369 "editosome" evolved into a concept of an RNA editing holoenzyme. For the purposes of this
370 review, we shall equate the editosome and editing holoenzyme and define this entity as an
371 RNA-mediated assembly of the RNA editing catalytic (RECC), RNA editing substrate
372 binding (RESC) and RNA editing helicase REH2 (REH2C) complexes. It is a virtual
373 certainty that additional components are also involved [19, 21, 119]. This definition stems
374 from parallel lines of inquiry by the Stuart, Afasizhev and Lukeš laboratories that identified
375 an ~800 kDa protein complex (originally termed Mitochondrial RNA Binding Complex 1,
376 MRB1, and Guide RNA Binding Complex, GRBC), of which two components are essential
377 for gRNA stability [120-122]. Initially named GRBC1 and GRBC2, these homologous
378 polypeptides lack annotated motifs and similarity to any protein outside of Kinetoplastids
379 [63]. GRBC1 and GRBC2, also referred to as GAP2 and GAP1, respectively [122], form a
380 stable heterotetramer which binds gRNA *in vitro* and *in vivo* [52]. Extensive co-purification
381 and yeast two-hybrid screens further dissected MRB1 into two relatively stable protein
382 complexes: an ~20-component RNA Editing Substrate Binding Complex (RESC), which
383 includes RESC1 (GRBC1, GAP2) and RESC2 (GRBC2, GAP1), and three-subunit RNA
384 Editing Helicase 2 Complex (REH2C) (Table 1). It appears that both RESC and REH2C bind
385 editing substrates, intermediates and products, and engage in RNA-mediated interactions with
386 the catalytic RECC complex [52, 123-126]. All but five of the RESC subunits lack
387 discernible motifs or similarities to non-kinetoplastid proteins, although several exhibit *in*
388 *vitro* RNA binding activity [19, 21, 119]. Most subunits are essential for cell viability and
389 their knockdowns typically produce phenotypes consistent with an inhibited editing process.
390 Recently, a substantial progress has been made in deciphering roles of individual factors. The

RESC1/2 tetramer appears to be solely responsible for gRNA stabilization [121, 122]. Deep sequencing studies showed that the RRM/RGG-containing RESC13 (RGG2) and proximal protein RESC11A (MRB8180) contribute to editing processivity within an extended domain [78, 127]. These two proteins strongly promote the formation of junctions, implying a critical role of these regions in editing progression [78]. Conversely, the product of a duplicate gene RESC12A (MRB8170) has been implicated in editing initiation and in constraining the region of active editing [78, 128]. Biochemical attempts to refine RESC architecture indicate a modular organization with potential protein clusters responsible for interaction with the RECC and polyadenylation complexes [52, 129]. However, an unambiguous assignment of specific polypeptides to functionally meaningful modules awaits elucidation of a high-resolution structure of the RESC complex, which is likely to be heterogenous and dynamic.

RNA editing is an essential processing step for a subset of mitochondrial transcripts and must be integrated into a general pathway of producing translation-competent mRNAs. Accumulating evidence suggests that the RESC is responsible for coordinating pre- and post-editing processing events via RNA-mediated contacts with 5′ and 3′ modification complexes, and auxiliary factors. On the other hand, the catalytic RECC isoforms appear to act on RESC-bound editing substrates in a transient manner. The RNA-mediated interaction between RESC and PPsome has been deduced from co-purification of RESC1/2 (GRBC1/2) and MERS1 hydrolase [23, 121, 130] whereas *in vivo* proximity biotinylation identified RESC19 (MERS3) as the most plausible adapter subunit [23]. An independent study predicted Z-DNA-binding domains in RESC19 (termed RBP7910) and showed that *in vitro* this protein preferentially binds RNAs containing poly(U) and poly(A/U)-rich sequences [131]. Likewise, kinetoplast polyadenylation complex components have been consistently detected in various RESC preparations and particularly in those with tagged RESC15-18 [29, 32, 52]. Furthermore, RESC13 (RGG2) and surrounding proteins likely mediate contacts between

RESC and RECC complexes [52]. Finally, RNA editing helicase 2 (KREH2, see below) preferentially associates with RESC variants purified by tagging of either RESC1 or RESC2 [52, 121, 132-134]. Thus, the RESC complex functions not only in binding of RNA editing substrates, intermediates and products, but also recruits mRNA modification complexes and auxiliary factors via specific subunits.

The Kinetoplast RNA Editing Helicase 2 (KREH2) complex, termed REH2C, consists of DEAH/RHA RNA helicase KREH2, and KREH2-associated factors 1 (KH2F1) and 2 (KH2F2). KH2F1contains eight C2H2 zing fingers while KH2F2 lacks any identifiable motifs [135] (Table 1). Isolated REH2C exhibits ATP-dependent 3′-5′ dsRNA unwinding activity and co-sediments with a major peak of same activity in mitochondrial extracts [134]. Zinc finger protein KH2F1 emerged as an adaptor connecting KREH2 helicase with the editosome while gRNA-mRNA hybridization has also been implicated in facilitating this interaction [135]. KREH2 and KH2F1 knockdowns display consistent phenotypes of increased editing pausing and reduced processivity of editing, which are indicative of REH2C participation in continuous remodeling of gRNA-mRNA contacts and perhaps the entire editosome [123, 135]. It must be emphasized that the reciprocal affinity purifications remain the most salient evidence of the editosome being an RNA-based assembly of RECC, RESC and REH2C protein complexes [120-122, 133, 134].

Auxiliary RNA Processing Factors

Putative Poly(A) Polymerase KPAP2

A putative kinetoplast poly(A) polymerase KPAP2 has been identified by homology to the human mitochondrial enzyme and apparently is not required for axenic *T. brucei* growth in either bloodstream or procyclic life stages [136]. Although KPAP2 protein sequence is highly

similar to that of KPAP1, its enzymatic identity and *in vivo* substrates remain to be established. Available proteomics data do not support KPAP2 association with KPAC [29, 31, 32].

REH1 RNA Helicase

Editing reactions are expected to produce an mRNA-gRNA duplex wherein gRNA must be eventually displaced to allow binding of a sequential gRNA, or before the edited transcript can be translated. It stands to reason that active remodelers, such as DEAD/H-box RNA helicases, would be involved and indeed two such proteins have been implicated in the editing process. However, demonstrating their specific roles, RNA targets and mechanism of action proved to be challenging. Knockdown of KREH1 (Hel61) helicase [137, 138] affected editing mediated by two or more overlapping gRNAs [139], but the existing data do not allow unambiguous placing of KREH1 into mRNA editing or processing complexes. The nature of KREH1 substrates and the timing and purpose of KREH1-dependent RNA remodeling also remain unclear. Although much remains to be understood about the helicases' involvement in editing and other processes, the transcript-specific impacts of repressing KREH1 and KREH2 (discussed above) justify further efforts to dissect their molecular functions.

RNA Binding Factors

Kinetoplast Mitochondrial RNA Binding Proteins 1 and 2 (KMRP1 and 2), originally called gBP21 and gBR25, then MRP1 and MRP2, were identified independently in *T. brucei* by UV-induced crosslinking with synthetic gRNA (gBP21, [140]), in *C. fasciculata* as poly(U) binding proteins (gBP21 and gBP25, [141]), and in *L. tarentolae* via crosslinking to double-stranded RNA resembling the U-deletion site (MRP1 and MRP2, [130]). Extensive biochemical and structural studies concluded that KMRP1 and KMRP2 assemble into an

~100 kDa heterotetramer, which binds both single- and double-stranded RNAs with high affinity [130, 142, 143]. These RNA binding properties are manifested by an RNA annealing activity, an attractive accessory function that may promote gRNA binding to cognate mRNA targets [144, 145]. However, the transcript-specific impact of dual KMRP1/2 repression suggests a contribution to stabilization of moderately edited and some never-edited mRNAs rather than direct participation in the editing process [142, 146, 147]. In support of this notion, RNAi experiments demonstrated that MRP1/2 depletion virtually eliminates the edited form of the moderately-edited *cyb* mRNA, but exerts little impact on the pre-edited transcript [146-148]. While much is known about the KMRP1/2 structure and *in vitro* properties, the definitive function of this RNA binding complex remains to be established. Much of the same narrative applies to KRBP16 (RBP16), which carries N-terminal cold shock and C-terminal RG-rich domains [149]. RNAi studies revealed an overlap between mRNA sets negatively affected by individual KMRP1/2 and KRBP16 knockdowns: edited *cyb* mRNA, but not any other edited transcripts, was severely downregulated while never-edited CO1 and ND4 transcripts also declined [146]. KRBP16 *in vitro* properties, such as RNA binding affinity, RNA annealing activity and stimulation of editing activity [146, 148-155], and the impact of RNAi knockdown on the initiation of *cyb* mRNA editing [148] are consistent with participation in the editing process, although the mechanistic role remains to be firmly established. Another enigmatic RNA binding protein, KRGG1 was serendipitously discovered in a large (>50S) RNP of unknown nature [156], and subsequently demonstrated to associate with the RESC complex [122, 157]. A different study identified a ribosome-bound KRGG1 fraction, which would explain the observed sedimentation patterns, but found no impact on RNA editing [52]. Another arginine-glycine-rich protein KRGG3, originally identified by association with RESC1/2 proteins and termed MRB1820 [129], is essential for parasite viability [158]. However, most KRGG3's interactions appear to be RNA-mediated

while the RNAi knockdown does not significantly impact major mitochondrial RNA classes. A structural study identified an ABC-ATPase fold and potential RNA binding surface in a 72 kDa protein KRBP72, initially termed MRB1590 [159]. KRBP72 knockdown specifically impacts editing of A6 mRNA [159]; however, an unequivocal functional placement of this factor also requires further investigation. Finally, participation of KREAP1 in editing [160, 161] has been contravened by a report of general mitochondrial RNA upregulation upon its knockdown and non-essentiality for parasite survival [162]. Mitochondrial RNA binding proteins are abundant and notoriously promiscuous in their interactions and pleiotropic effects on RNA steady-state levels [163], which makes an unequivocal definition of their function a challenge worth meeting.

Ribonucleases

Mitochondrial RNA processing most likely involves nucleolytic events beyond mRNA cleavage by editing KREN1, 2 and 3 endonucleases, KREX1 exonuclease, and 3′-5′ degradation by the MPsome-embedded KDSS1. To that end, three distinct enzymes have been identified and characterized to various degrees. The single-strand uridine specific KRND1 3′-5′ exonuclease [164] displays *in vitro* specificity for single-stranded uridines, similar to that of KREX1 editing enzyme [108, 110], and yet possesses an RND rather than EEP exonuclease domain. Given the diversity of U-tailed RNAs in the kinetoplastid mitochondrion, it is tempting to speculate on KRND1 involvement in regulating the 3′ modification state, but its definitive function remains to be established. The same narrative applies to KRPN1, an RNase III-like endonuclease with a characteristic double-stranded RNA cleaving activity [42, 46] suggested to function in gRNA processing. Further studies are required to reconcile KRPN1 activity and interactions with an exonucleolytic mechanism of gRNA precursor processing by the MPsome. Finally, the discovery of PPR-repeat containing proteinaceous RNase P (PROPR2 [165], re-named here as KRNP1) supported earlier reports

514 of RNase P activity-like which removes the 5′ leader from a synthetic tRNA precursor in
515 mitochondrial lysate [166]. However, tRNAs are apparently imported into the mitochondrion
516 with 5′ and 3′ extensions removed [167, 168], which leaves the nature of KRNP1 *in vivo*
517 substrates open to future inquiry.

518

519 ## Concluding Remarks

520 This review compiles 74 processing enzymes, RNA binding proteins and factors with
521 unknown functionality that nonetheless are associated with RNA processing complexes.
522 Proteomics and interactions analyses allowed clustering most of these into 5-6
523 macromolecular assemblies albeit with various degree of confidence. Although the list is
524 almost certainly incomplete, with complexes and individual proteins, and their interactions
525 and functions being constantly re-examined, the overall flow of RNA processing in
526 trypanosome mitochondrion is taking shape and meaning. The key players responsible for
527 maturation of 5′ and 3′ termini have been defined and initial insights gained into the
528 molecular mechanism of internal sequence changes by editing. At this point, we suggest that
529 the RNA editing holoenzyme (editosome) represents an RNP that chiefly includes three
530 relatively stable protein complexes (RECC, RESC and REH2C) and RNA editing substrates,
531 intermediates and products. It is understood that the definition of protein complex is to large
532 extent a matter of purification techniques and we posit that future structural studies will shed
533 light on stoichiometry and functions of individual subunits and modules. Because of
534 sequence changes introduced by editing during mRNA processing, the overall picture is
535 emerging of the mRNA fate being dictated by diverse PPR RNA surveillance factors. These
536 proteins direct 5′ pyrophosphate removal, transcript stabilization and pre-editing A-tailing,
537 monitor initiation and progression of editing, and signal its completion by stimulating the

A/U-tailing. Displacement of bound PPRs from pre-edited mRNA by the editing process appears to be the principal quality control mechanism. It remains to be established whether active RNP remodeling takes place or sequence changes alone suffice for this purpose. In any event, the plurality of PPRs and their capacity to read linear sequences and modulate the activity of RNA modification and degradation enzymes position this protein family as the focal point of mitochondrial RNA processing.

Acknowledgements

Afasizheva lab is supported by NIH grant AI113157. Afasizhev lab is supported by NIH grants AI091914 and AI101057. Cruz-Reyes lab is supported by NSF grant 1616845 and TAMU X-grant and T3 award. Göringer lab is supported by German Research Council (SPP1857). Lukeš lab is supported by the Czech Ministry of Education (OPVVV16_019/0000759 and ERC CZ LL1601. Read lab is supported by NIH grants AI061580, GM129041, and AI139448. Hajduk lab is supported by NIH grant AI125487. Schnaufer lab is supported by MRC Senior Non-Clinical Fellowship MR/L019701/1. Schneider lab is supported by the NCCR grant "RNA & Disease" and by the grant 175563 from the Swiss National Science Foundation. Stuart lab is supported by NIH grant 5R01AI014102-43. Yurchenko lab is supported by the European Regional Funds (project "Centre for Research of Pathogenicity and Virulence of Parasites", OPVVV16_ 019/0000759). Zíková lab is supported by the Czech Ministry of Education (OPVVV16_019/0000759) and by GACR grant 18-17529S. Zimmer lab is supported by NIH grant AI135885.

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Glossary

5′ and 3′ cleavage fragments: mRNA fragments generated by gRNA-directed endonucleolytic cleavage.

Anchor: 5′ part of the gRNA that forms a continuous 10-15 nt duplex with pre-edited, partially-edited or fully-edited mRNA; this region is responsible for initial gRNA-mRNA interaction.

Cryptogene: a maxicircle gene with defective coding sequence; the defects are corrected by U-insertion/deletion editing with concomitant restoration of protein reading frame.

Editing block: an mRNA segment covered by a single gRNA; often contains both U-insertion and U-deletion sites.

Editing domain: an mRNA region covered by single or multiple overlapping gRNAs. In a multi-gRNA domain, sequence changes directed by the initiating gRNA create the binding site for the subsequent one. The hierarchical gRNA binding provides for the overall 3′-5′ progression of editing events within a domain.

Editing site: position of the gRNA-directed internal cleavage where uridines are either deleted from or inserted into the mRNA.

EEP domain: endonuclease/exonuclease/phosphatase (EEP) domain in U-specific editing exonucleases.

Fully-edited mRNA: a final editing product; contains a protein coding frame.

Guide RNA (gRNA): a small non-coding RNA that specifies positions and extent of U-insertions and deletions by forming an imperfect duplex with pre-edited or partially-edited mRNA. Guide RNA is typically 30-60 nucleotides (nt) in length and possesses a 5′ triphosphate and a 1-20 nt 3′ U-tail.

Junction: A region present in most partially-edited mRNAs at the 5′ leading edge of editing; often displays mis-edited and non-canonically edited sequences. Junction-containing transcripts may represent intermediates that will be re-edited to canonical sequence, dead-end by-products, and mRNAs with a non-canonical protein coding sequence.

Kinetoplastids (class Kinetoplastea): flagellated protists characterized by the presence of a kinetoplast. Phylogenetically positioned within the phylum Euglenozoa, this group includes the obligatory parasitic trypanosomatids (family *Trypanosomatidae*), free-living and parasitic bodonids, and more distantly related taxa.

Kinetoplast: a densely packed nucleoprotein structure, disc-shaped and catenated in trypanosomatids, and dispersed to various degrees in most bodonids, that encloses mitochondrial DNA (kinetoplast DNA; kDNA). A non-dividing *T. brucei* cell contains a single mitochondrion with a single kinetoplast composed of catenated maxicircles (~23 kb each, few dozen copies) and minicircles (~1 kb each, ~5,000 units).

KPAC: kinetoplast polyadenylation complex. A ribonucleoprotein complex of KPAP1 poly(A) polymerase, and pentatricopeptide repeat-containing (PPR) RNA binding proteins designated Kinetoplast Polyadenylation Factors 1, 2, 3 and 4 (KPAF1, 2, 3 and 4).

Maxicircle: an equivalent of a typical mitochondrial genome; includes a conserved ~15 kb region encoding 9S and 12S rRNAs, two guide (g)RNAs, and 18 protein genes. A variable region composed of repeated DNA sequences constitutes the rest of the molecule.

Minicircle: the molecules forming the bulk of kinetoplast. Approximately 400 sequence classes present at various frequencies encode ~930 gRNAs required for the editing process and 370 gRNA-like molecules that likely participate in gRNA processing.

Moderately-edited mRNA: a transcript with a few editing sites confined to a limited mRNA region.

MPsome: mitochondrial 3′ processome. A protein complex composed of Kinetoplast RNA editing TUTase 1 (KRET1), 3′-5′ exonuclease KDSS1, and MPSS1-6 subunits lacking recognizable motifs.

MTRNAP: mitochondrial RNA polymerase, a single-subunit T3/T7-like DNA-dependent RNA polymerase.

Never-edited mRNA: a maxicircle transcript containing an encoded open reading frame which does not require editing.

Pan-edited mRNA: a transcript that undergoes massive editing directed by multiple gRNAs. There can be two editing domains within a pan-edited mRNA.

Partially-edited mRNA: an intermediate of the editing process. Partially-edited mRNAs often contain junctions whose sequences match neither pre-edited nor canonical fully-edited mRNAs.

PPsome: 5′ pyrophosphate processome. Protein complex containing MERS1 NUDIX pyrophosphohydrolase and MERS2 PPR RNA binding protein.

PPR: Pentatricopeptide (35 amino acids) helix-turn-helix repeat. PPR arrays are present in many trypanosomal mitochondrial RNA binding proteins.

Pre-edited mRNA: a 3′ processed monocistronic cryptogene transcript that must undergo editing to acquire an open reading frame and/or translation initiation and termination signals.

RECC: RNA Editing Catalytic Complex, formerly called ~20S editosome or RNA Editing Core Complex. A protein complex of 14 or more subunits, depending on the isoform; includes pre-mRNA cleavage, U-insertion, U-deletion and RNA ligation enzymes, and structural and RNA binding factors.

RESC: RNA Editing Substrate Binding Complex, formerly called Mitochondrial RNA Binding Complex 1 (MRB1) and Guide RNA Binding Complex (GRBC). An ~20-subunit modular protein complex that likely exists in several isoforms; most components lack recognizable motifs. RESC binds RNA editing substrates, intermediates and products, and coordinates interactions of gRNA and mRNA with RECC, REH2C and other auxiliary factors during editing. RESC has also been implicated in coordination of pre-mRNA 5′ and 3′ modification processes.

REH2C: RNA Editing Helicase 2 Complex, a protein complex formed by an ATP-dependent DEAH/RHA RNA helicase KREH2, zinc finger protein KH2F1, and KH2F2 factor which lacks recognizable motifs.

RNA editing holoenzyme (editosome): a ribonucleoprotein particle consisting of RECC, RESC and REH2C complexes, and several auxiliary factors.

RNA helicase: a motor protein capable of harnessing the energy from NTP hydrolysis to unwind double stranded RNAs or to remodel ribonucleoprotein complexes.

RNase II: Exoribonuclease II cleaves single-stranded RNA in 3' to 5'-direction yielding nucleoside 5′ monophosphates.

RNase III: Endoribonuclease III typically cleaves both strands in double-stranded RNA leaving 5′ monophosphate and 3′ hydroxyl groups. RNase III editing endonucleases cleave only the mRNA strand at an unpaired nucleotide adjacent to a gRNA-mRNA duplex.

TUTase: terminal uridyltransferase, UTP-specific nucleotidyl transferase which adds U-residues to the 3′ end of RNA.

U-insertion/deletion mRNA editing: a process by which U-residues are inserted into, or deleted from, a cryptogene transcript. Editing is directed by gRNAs and catalyzed by the RNA editing holoenzyme (editosome).

UTR: untranslated region of mRNA.

1086 **Table 1.** Proposed nomenclature of mitochondrial RNA processing complexes and factors.

1087 Gene identification numbers refer to *T. brucei* strain TREU927 predicted protein sequences

1088 (TriTrypDB, Release 45, September 5, 2019, https://tritrypdb.org/tritrypdb/).

Legacy		Assigned	Function	Motifs	TriTryp ID	References
Nucleolytic Processing: Mitochondrial 3′ processome (MPsome)						
RET1	KRET1	**KRET1**	TUTase, 3′ uridylation of primary and mature RNAs	TUTase, PAP associated	Tb927.7.3950	[24, 25, 48, 49, 169]
KDSS1	KDSS1	**KDSS1**	3′-5′ exonuclease	RNB (Ribonuclease II)	Tb927.9.7210	[24, 26, 50]
MPSS1		**MPSS1**			Tb927.11.9150	[24]
MPSS2		**MPSS2**			Tb927.10.9000	[24]
MPSS3		**MPSS3**			Tb927.3.2770	[24]
MPSS4		**MPSS4**			Tb927.10.6170	[24]
MPSS5		**MPSS5**			Tb927.9.4810	[24]
MPSS6		**MPSS6**			Tb927.6.2190	[24]
Modification of the 5′ end: Pyrophosphohydrolase complex (PPsome)						
MERS1		**MERS1**	PPi removal from 5′ end	NUDIX hydrolase	Tb927.11.15640	[23, 121, 122]
MERS2		**MERS2**	Targets MERS1 to RNA	PPR	Tb11.02.5120	[23]
Modification of the 3′ end: Kinetoplast polyadenylation complex (KPAC)						
KPAP1		**KPAP1**	Major poly(A) polymerase	NT/TUTase, PAP associated	Tb927.11.7960	[31]
KPAF1	PPR1	**KPAF1**	mRNA A/U-tailing	PPR	Tb927.2.3180	[30, 170, 171]
KPAF2		**KPAF2**	mRNA A/U-tailing	PPR	Tb927.11.14380	[30]
KPAF3		**KPAF3**	mRNA stabilization/A-tailing	PPR	Tb927.9.12770	[29]
KPAF4		**KPAF4**	Poly(A) binding protein	PPR	Tb927.10.10160	[32]
U-insertion/deletion mRNA Editing: RNA editing catalytic complex (RECC)						
REN1	KREPB1	**KREN1**	U-deletion endonuclease	RNase III, PUF, ZF-C2H2	Tb927.1.1690	[86, 172]
REN2	KREPB3	**KREN2**	U-insertion endonuclease	RNase III, PUF, ZF-C2H2	Tb927.10.5440	[86, 173]
REN3	KREPB2	**KREN3**	U-insertion endonuclease	RNase III, PUF, ZF-C2H2	Tb927.10.5320	[85, 86, 98]
REX1	KREX1	**KREX1**	3′-5′ U-specific exonuclease	Exo/endo/phos (EEP)	Tb927.7.1070	[85, 86, 97]
REX2	KREX2	**KREX2**	3′-5′ U-specific exonuclease	Exo/endo/phos (EEP)	Tb927.10.3570	[85, 86, 97]
RET2	KRET2	**KRET2**	U-insertion TUTase	TUTase, PAP associated	Tb927.7.1550	[48, 85, 86, 113]
REL1	KREL1	**KREL1**	RNA ligase (U-deletion)	RNA lig/RNL2	Tb927.9.4360	[47, 85, 86, 90]
REL2	KREL2	**KREL2**	RNA ligase (U-insertion)	RNA lig/RNL2	Tb927.1.3030	[85, 86, 90]
MP81	KREPA1	**KREPA1**		ZF-C2H2, OB fold	Tb927.2.2470	[85, 86, 174]
MP63	KREPA2	**KREPA2**		ZF-C2H2, OB fold	Tb927.10.8210	[85, 86, 175]
MP42	KREPA3	**KREPA3**		ZF-C2H2, OB fold	Tb927.8.620	[85, 86, 92, 176]
MP24	KREPA4	**KREPA4**		OB fold	Tb927.10.5110	[85, 86, 94]
MP19	KREPA5	**KREPA5**		OB fold	Tb927.8.680	[86]
MP18	KREPA6	**KREPA6**		OB fold	Tb927.10.5120	[85, 86, 91, 177]
MP46	KREPB4	**KREPB4**		RNase III, PUF, ZF-C2H2	Tb927.11.2990	[85, 86, 103]
MP44	KREPB5	**KREPB5**		RNase III, PUF, ZF-C2H2	Tb927.11.940	[85, 86, 176, 178]
MP49	KREPB6	**KREPB6**		RNase III, ZF-C2H2	Tb927.3.3990	[85, 86, 95, 102]
MP47	KREPB7	**KREPB7**		RNase III, ZF-C2H2	Tb927.9.5630	[95, 102, 104]
MP41	KREPB8	**KREPB8**		RNase III, ZF-C2H2	Tb927.8.5690	[95, 102, 104]
	KREPB9	**KREPB9**		RNase III, ZF-C2H2	Tb927.9.4440	[179, 180]
	KREPB10	**KREPB10**		RNase III, ZF-C2H2	Tb927.8.5700	[179, 180]
MEAT1		**MEAT1**	RECC-like associated TUTase	TUTase, PAP associated	Tb927.1.1330	[181]

U-insertion/deletion mRNA Editing: RNA editing substrate binding complex (RESC)						
GRBC1	GAP2	**RESC1**	gRNA binding/stabilization		Tb927.7.2570	[121, 122]
GRBC2	GAP1	**RESC2**	gRNA binding/stabilization		Tb927.2.3800	[121, 122]
GRBC3	MRB8620	**RESC3**			Tb927.11.16860	[52, 124, 129]
GRBC4	MRB5390	**RESC4**			Tb11.02.5390b	[52, 129, 182]
GRBC5	MRB11870	**RESC5**			Tb927.10.11870	[52, 129, 183]
GRBC6	MRB3010	**RESC6**			Tb927.5.3010	[52, 123, 129, 133, 184]
GRBC7	MRB0880	**RESC7**			Tb927.11.9140	[52, 129]
REMC1	MRB10130	**RESC8**	RNA binding	ARM/HEAT repeats	Tb927.10.10130	[52, 120, 125, 129]
REMC2	MRB1860	**RESC9**			Tb927.2.1860	[52, 129]
REMC3	MRB800	**RESC10**			Tb927.7.800	[52, 129]
REMC4	MRB8180 MRB4150	**RESC11A** **RESC11B**	RNA binding		Tb927.8.8180 Tb927.4.4150	[52, 78, 129]
REMC5	MRB4160	**RESC12**	RNA binding		Tb927.4.4160	[52, 128, 129, 185]
REMC5A	MRB8170	**RESC12A**			Tb927.8.8170	[52, 78, 128, 129, 185]
TbRGG2	TbRGG2	**RESC13**	RNA binding	RGG, RRM	Tb927.10.10830	[52, 78, 127, 129, 182, 186-188]
	MRB7260	**RESC14**		PhyH	Tb927.9.7260	[126, 129]
PAMC1		**RESC15**			Tb927.1.1730	[52]
PAMC2		**RESC16**			Tb927.6.1200	[52]
PAMC3		**RESC17**			Tb927.10.1730	[52]
PAMC4		**RESC18**			Tb927.1.3010	[52]
MERS3	RBP7910	**RESC19**		Z-DNA binding	Tb927.10.7910	[23, 131]
U-insertion/deletion mRNA Editing: REH2 RNA Helicase Complex (REH2C)						
REH2		**KREH2**	RNA helicase, RNA binding	DEAH/RHA, HA2, DSRM, OB/NTP_binding	Tb927.4.1500	[120-123, 132-135]
H2F1	MRB1680	**KH2F1**		ZF-C2H2	Tb927.6.1680	[134, 135, 182]
H2F2		**KH2F2**			Tb927.6.2140	[132, 134, 135]
Auxiliary RNA Processing Factors						
KPAP2		**KPAP2**	Putative poly(A) polymerase	NT/TUTase, PAP associated	Tb927.10.160	[136]
REH1	mHEL61	**KREH1**	RNA helicase	DEAD/DEAH box helicase	Tb927.11.8870	[139, 189]
MRP1	gBP21	**KMRP1**	RNA binding		Tb927.11.1710	[130, 140-148]
MRP2	gBP25	**KMRP2**	RNA binding		Tb927.11.13280	[130, 142, 143, 146-148]
RGG1		**KRGG1**	RNA binding		Tb927.6.2230	[122, 156, 157]
RBP16		**KRBP16**	RNA binding	Cold-shock RNA binding	Tb927.11.7900	[146, 148-155]
	MRB1590	**KRBP72**	RNA binding	ABC-like ATPase domain	Tb927.3.1590	[159]
TbRGG3	MRB1820	**KRGG3**			Tb927.3.1820	[129, 158]
REAP-1		**KREAP1**	RNA binding		Tb927.10.9720	[160-162]
RND		**KRND1**	U-specific 3′-5′ exonuclease	RND, ZF-C2H2	Tb927.9.12720	[164]
PRORP2		**KRNP1**	RNase P	PRORP, PPR	Tb927.11.3010	[165]
mRPN1		**KRPN1**	Endonuclease	RNase III	Tb927.11.8400	[42, 46]

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Figure 1. A schematic diagram of mitochondrial RNA processing in *T. brucei.* The flow of processing reactions does not imply an experimentally established timing of these events. For example, the rRNA assembly into the ribosome or 5′ pyrophosphate removal from mRNA may occur co-transcriptionally. Likewise, the mRNA may be edited prior to completion of 3′-5′ trimming and 3′ adenylation.

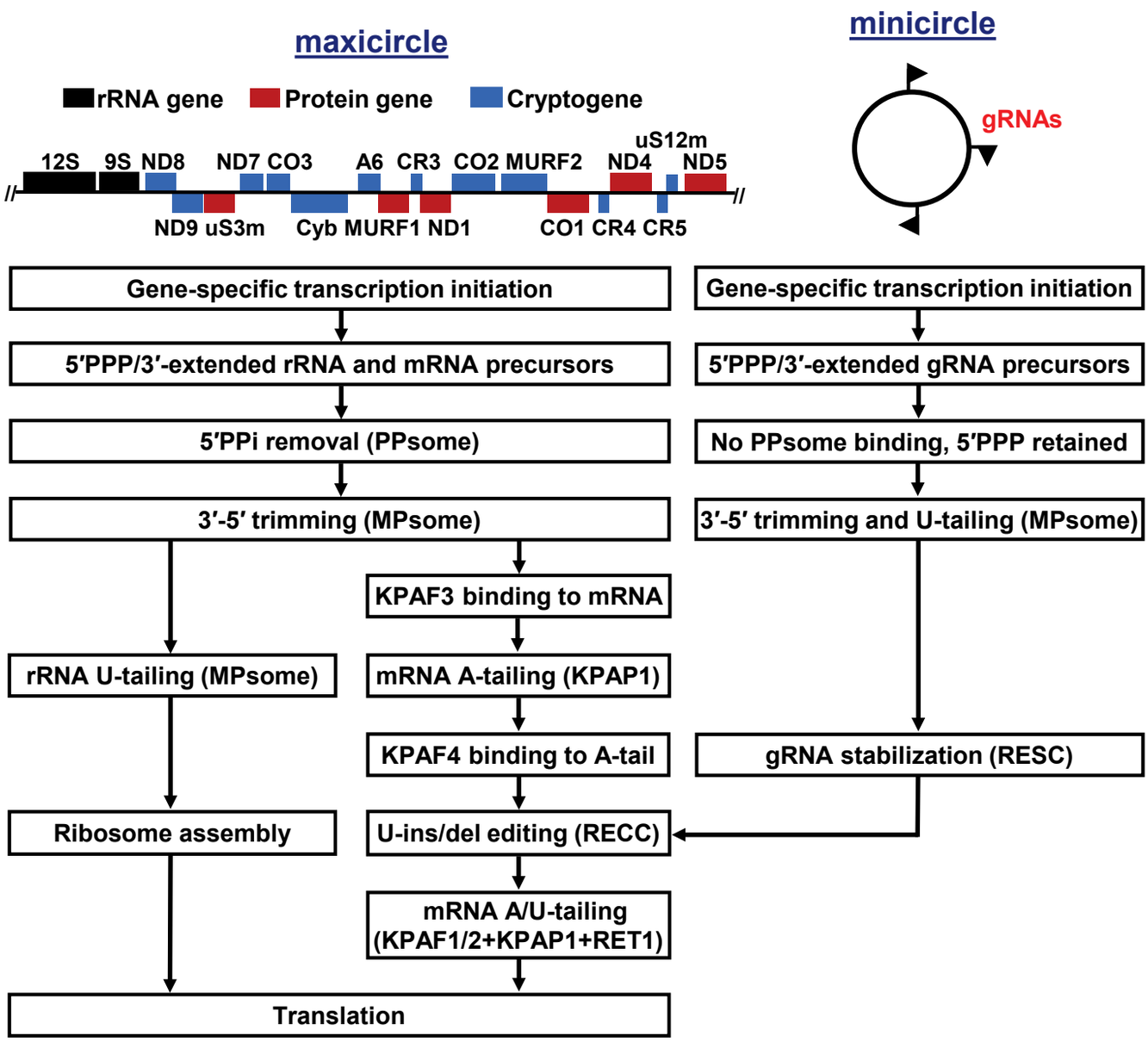

Fig. 1